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IS 2237 (1997): Prawns (Shrimps) - Frozen [FAD 12: Fish and Fisheries Products]

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झींगा (श्रिम्प) — फ्रोजन — विशिष्टि

(तीसरा पुनरीक्षण)

Indian Standard

PRAWNS (SHRIMPS) — FROZEN — SPECIFICATION

(Third Revision)

ICS 67.120.30

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BUREAU OF INDIAN STANDARDS
MANAK BHAVAN, 9 BAHADUR SHAH ZAFAR MARG
NEW DELHI 110002

October 1997 Price Group 7

AMENDMENT NO. 1 JULY 2005
TO
IS 2237 : 1997 PRAWNS (SHRIMPS) — FROZEN — SPECIFICATION

(*Third Revision*)

[*Page* 1, *clause* 2, *para* 2] — Substitute the following for the existing matter against relevant IS Numbers:

Sl No.	*Title*
5887 (Part 7) : 1999	Methods for detection of bacteria responsible for food poisoning : Part 7 General guidance on methods for isolation and identification of *Shigella*
11427 : 2001	Fish and fishery products — Sampling (*first revision*)

[*Page* 3, *Table* 2, *Sl No.* (i), *col* 4] — Substitute '500 000' *for* '1 000 000'.

[*Page* 3, *Table* 2, *Sl No.* (iii), *col* 5 *and* 6] — Substitute '100' *for* '*Absent*'.

[*Page* 3, *Table* 2, *Sl No.* (vi), *col* 7] — Substitute 'IS 5887 (Part 7)' *for* 'IS 5887 (Part 3)'.

(FAD 12)

Reprography Unit, BIS, New Delhi, India

Fish and Fisheries Products Sectional Committee, FAD 12

FOREWORD

This Indian Standard (Third Revision) was adopted by the Bureau of Indian Standards, after the draft finalized by the Fish and Fisheries Products Sectional Committee had been approved by the Food and Agriculture Division Council.

Fish is one of the most perishable of all foods and needs proper care from the time it is caught until it is served or processed. Lowering the temperature of fish by a prompt and efficient chilling procedure is fundamental for preservation of fish freshness. The quality of frozen fishery products is influenced by many different considerations. Among the most important are composition of fish, pre-freezing, handling and transport, method of freezing employed and the environment to which the frozen product is subjected during storage and handling. Of principal concern here is the temperature and humidity of the cold storage area and the protective packaging or glazing afforded the product.

Frozen prawns constitute an important export commodity of India. This standard was first published in 1962 and underwent first revision in 1971 and second revision in 1985. This revision, incorporates among other the following changes:

i) Existing microbiological requirements have been modified along with incorporation of new microbiological requirements along with their methods of tests;

ii) For safeguarding the health of consumers, maximum permissible limits for heavy metals have been prescribed, along with their methods of tests; and

iii) Editorial changes includes updating of referred standards.

In the preparation of this standard, due consideration has been given to the provisions of the *Prevention of Food Adulteration Act*, 1954 and *Standards of Weights and Measures* (*Packaged Commodity*) *Rules*, 1977. However, this standard is subject to the restrictions imposed under these, wherever applicable.

For the purpose of deciding whether a particular requirement of this standard is complied with the final value, observed or calculated, expressing the result of a test or analysis, shall be rounded off in accordance with IS 2 : 1960 'Rules for rounding off numerical values (*revised*)'. The number of significant places retained in the rounded off value, should be the same as that of the specified value in this standard.

Indian Standard

PRAWNS (SHRIMPS) — FROZEN — SPECIFICATION

(Third Revision)

1 SCOPE

1.1 This standard prescribes the requirements and the methods of sampling and test for frozen prawns (shrimps).

1.1.1 The frozen prawns (shrimps) shall be of any edible species.

2 REFERENCES

The following Indian Standards listed contain provisions which through reference in this text, constitute provision of this standard. At the time of publication, the editions indicated were valid. All standards are subject to revision and parties to agreements based on this standard are encouraged to investigate the possibility of applying the most recent editions of the standards indicated:

IS No.	*Title*
1070 : 1992	Reagent grade water (*third revision*)
4303 (Part 1) : 1975	Code of hygienic condition for fish industry : Part 1 Preprocessing stage (*first revision*)
5887 (Part 3) : 1975	Methods for detection of bacteria responsible for food poisoning: Part 3 Isolation and identification of *Salmonella* and *Shigella* (*first revision*)
11427 : 1985	Methods for sampling for fish and fisheries products

3 TYPES

3.1 Frozen prawns shall be of the following types.

3.1.1 *Whole* — Head and shell on.

3.1.2 *Headless* — Head removed, shell on.

3.1.3 *Fantail Round* — Head and shell removed except on the last segment and tail.

3.1.4 *Fantail Deveined* — As in 3.1.3 but the dorsal tract removed.

3.1.5 *Fantail Butterfly* — As in 3.1.3 in addition to splitting open.

3.1.6 *Peeled and Deveined* — Head and shell and dorsal tract removed completely.

3.1.7 *Cooked and Peeled* — Peeled after cooking.

3.1.8 *Peeled Deveined and Cooked* — As in 3.1.6 but cooked.

3.1.9 *Whole, Cooked* — As in 3.1.1 but cooked.

3.1.10 *Peeled Undeveined* — Head and shell removed completely.

3.1.11 *Headless Blanched* — As in 3.1.2 but blanched.

3.1.12 *Peeled and Deveined Blanched* — As in 3.1.6 but blanched.

3.1.13 *Peeled Undeveined and Blanched* — As in 3.1.10 but blanched.

3.1.14 *Individually Quick Frozen* (IQF) — Any of the types mentioned above but quick frozen individually.

4 DESCRIPTION

The shell and flesh of frozen prawns shall have characteristic colour of the respective types and shall be free from any black discoloration. The meat shall be firm and consistent.

5 REQUIREMENTS

5.1 The material shall be prepared from clean, wholesome and fresh prawns, and shall not show any visible signs of spoilage. The material shall as far as possible be free from any discoloration, off-odours, dehydration, black spots, whole insects or its fragments, rodent droppings and any other foreign matter. However, the defects for various quality characteristics shall not exceed the tolerance limits given in Table 1.

5.2 The material shall be prepared and processed as given in Annex A, under hygienic conditions as prescribed in IS 4303 (Part 1).

Table 1 Tolerance Limits for Defects for Various Characteristics

(*Clause* 5.1)

Sl No.	Characteristic	Tolerance Limit	
		Headless Shell on Type (percent)	Other Types (percent)
(1)	(2)	(3)	(4)
i)	Deterioration with spoiled pieces	5	5
ii)	Discoloration of shell and meat	10	20
iii)	Black spot on shell and meat	3	2
iv)	Broken, damaged and soft shelled pieces	5	10
v)	Legs, bits of veins, loose shells, etc	2	5
vi)	Dehydration	15	15

5.3 The material shall conform to the requirements prescribed in Table 2.

6 PACKING AND MARKING

6.1 Packing

The material shall be packed in suitable container as agreed between the purchaser and the processor. In the absence of any such agreement the material shall be packed in containers which may withstand the stress and strain of transportation and can prevent deterioration during transportation and frozen storage. A layer of polyethylene shall be used between the material and the container when individually frozen prawns are packed.

6.2 Marking

6.2.1 Each container shall be marked legibly and indelibly with the following information:

a) Name and type of the material with brand name, if any;

b) Name and address of the processor;

c) Batch or code number;

d) Count per kg (if required by the purchaser);

e) Minimum net mass of the contents which is equivalent to the drained mass ascertained after complete thawing and excluding the glaze;

f) Name of the additives used, if any;

g) Any other requirement as given under the *Standards of Weights and Measures (Packaged Commodities) Rules*, 1977.

6.2.2 *BIS Certification Marking*

The product may also be marked with the Standard Mark.

6.2.2.1 The use of the Standard mark is governed by the provisions of *Bureau of Indian Standards Act*, 1986 and the Rules and Regulations made thereunder. The details of conditions under which the licence for the use of Standard Mark may be granted to manufacturers or producers may be obtained from the Bureau of Indian Standards.

7 SAMPLING

The methods of drawing representative samples of the material for test and criteria for conformity shall be as prescribed in IS 11427.

NOTE — For determining the defects in cooked as well as raw frozen products, remove the wrapper of the frozen material and place it in a closed polythene bag. The polythene bag is further immersed in potable water at room temperature in a clean container wherein the potable water is introduced from the bottom of the container at a flow rate of 25 l/min. The material shall not come in direct contact with water. After all the glaze that can be seen or felt is removed, and the pieces separate easily, transfer the products to the preweighed sieve. Drain for 2 minutes at the inclined position and weigh. Calculate the net mass. Number of pieces of shrimp may be determined in the above material by counting the pieces in 1 kg.

Table 2 Requirement For Prawns (Shrimps) — Frozen

(*Clause* 5.3)

Sl No.	**Characteristic**	**Requirement**				**Method of Test, Ref to Annex and Other Indian Standard**
		Whole, Headless and IQF Type	Peeled and Deveined Type Including Butterfly, Fantail Round and IQF Type	Cooked Type Including Cooked-Peeled, Whole Cooked, Peeled-Deveined, Cooked and IQF	Blanched Type Including Headless Blanched, Peeled-Deveined Blanched, and Peeled Undeveined Blanched and IQF Type	
(1)	(2)	(3)	(4)	(5)	(6)	(7)
i)	Total bacterial count/g in the finished product, *Max*	500 000	1 000 000	100 000	200 000	B
ii)	*Escherichia coli* Count/g, *Max*	20	20	Absent	Absent	C
iii)	Faecal *Streptococci* count/g, *Max*	100	100	Absent	Absent	D
iv)	Coagulase positive *Staphylococci*/g, *Max*	100	100	Absent	Absent	E
v)	*Salmonella*/*Arizona*	Absent per 25 g	Absent per 25 g	Absent per 25 g	Absent per 25 g	F
vi)	*Shigella*	Absent per 25 g	Absent per 25 g	Absent per 25 g	Absent per 25 g	IS 5887 (Part 3)
vii)	*Vibrio cholerae*	Absent per 25 g	Absent per 25 g	Absent per 25 g	Absent per 25 g	G
viii)	*Listeria monocytogenes*	Absent per 25 g	Absent per 25 g	Absent per 25 g	Absent per 25 g	H
ix)	Formaldehyde mg/kg, *Max*	10.0	10.0	10.0	10.0	J
x)	Indole, mg/kg, *Max*	2.5	2.5	2.5	2.5	K
xi)	Heavy Metals :					
	a) Mercury, mg/kg, *Max*	0.5	0.5	0.5	0.5	L-3
	b) Copper, mg/kg, *Max*	20.0	20.0	20.0	20.0	L-4
	c) Zinc, mg/kg, *Max*	50.0	50.0	50.0	50.0	L-4
	d) Arsenic, mg/kg, *Max*	1.1	1.1	1.1	1.1	L-5
	e) Lead, mg/kg, *Max*	1.0	1.0	1.0	1.0	L-6
	f) Tin, mg/kg, *Max*	250.0	250.0	250.0	250.0	L-7

ANNEX A

(*Clause* 5.2)

PROCESSING OF PRAWNS (SHRIMPS) — FROZEN

A-1 Processing

A-1.1 The prawns shall be iced and maintained at a temperature of 3°C till they reach the factory for freezing.

A-1.2 The material along with crushed ice, shall be kept in clean stainless steel, aluminum or plastic containers of suitable size. It shall be processed, that is, converted into required product such as headless (deheaded), peeled and deveined or peeled and undeveined, blanched, etc.

A-1.2.1 For blanched products, the material shall be processed in stainless steel containers, heated electrically/or by steam by holding the product in water with or without additives or exposing to steam for required short period just to remove slime and/or bring about desirable change in colour of shell or meat but taking care that the product is not cooked. Best results can be obtained by immediate cooling in crushed ice or chilled water subsequent to blanching.

A-1.3 The material shall be size graded, and washed with chlorinated water containing 10 mg/kg available chlorine. It shall then be weighed and filled in polythene bags or waxed laminated cartons with a lining of waterproof material. The material after washing and selecting may be arranged on a conveyor for freezing.

A-1.4 The material shall be quick frozen at a temperature not exceeding −40°C in the minimum possible time. During freezing, the time taken for the core of the material, 5 cm thick, to reach a temperature ranging from −18 to −20°C, shall be preferably $1\frac{1}{2}$h, but shall not exceed 3 h under any circumstances.

A-1.5 The quick frozen material shall be uniformly glazed if frozen in containers other than waxed cartons and packed in suitable receptacles. The packed material shall be immediately shifted to the cold storage and stored at a minimum temperature of −18°C.

A-1.5.1 Individually quick frozen products shall be glazed, preferably hardened, prior to packing, weighing and stored at a minimum temperature of −22°C.

NOTE — Glazing shall be done with ice cold potable water containing 5 to 10 mg/kg available chlorine. Glazed water can also be added to the material before freezing to produce uniform glaze. Citric acid, sugar and sodium chloride may be added to the glazing water up to levels of 0.2, 0.5 and 0.5 percent respectively. Permitted food colours and/or other permitted additives may be used in the case of cooked and peeled; devened and cooked; whole cooked and blanched.

A-1.6 Food colours and/or other food additives permissible under the *Prevention of Food Adulteration Act and Rules*, 1955 may be used in the case of cooked and peeled; deveined and cooked; whole cooked and blanched prawns.

ANNEX B

[*Table* 2, *Sl No.* (i)]

DETERMINATION OF TOTAL BACTERIAL COUNT

B-1 MEDIUM

B-1.1 Tryptone glucose beef extract agar with the following composition shall be used:

Beef extract	3 g
Dextrose	1 g
Tryptone	5 g
Agar	15 g
Distilled water (*see* IS 1070)	1 litre

B-1.1.1 Dissolve the agar by steaming. Dissolved the other ingredients mentioned above (**B-1.1**) in the molten agar. Adjust *p*H to 7.2. Filter through absorbent cotton and distribute in suitable quantities in glass containers. Sterilize in an autoclave at 121°C for 30 minutes.

B-1.2 Phosphate Buffer

B-1.2.1 *Stock Solution*

Dissolve 34 g of potassium dihydrogen phosphate in 500 ml distilled water (*see* IS 1070), adjust the *p*H to 7.2 and make up the volume to 1 000 ml with distilled water (*see* IS 1070).

B-1.2.2 *Solution for Use*

Add 1.25 ml of the stock solution to 1 000 ml of distilled water (*see* IS 1070). Distribute in glass containers as required for serial dilution. Sterilize in an autoclave at 121°C for 30 minutes.

B-2 PROCEDURE

B-2.1 Collection of Sample

Scrap off the surface glaze from the material with a sterile scalpel. Using a sterile core sampler, in the case of blocks and sterile scissors, in the case of individual quick frozen materials, collect sample from the different parts of the frozen material to make up a total of about 10 g.

B-2.2 Preparation of Homogenate

Disintegrate the sample (B-2.1) with 90 ml of phosphate buffer (B-1.2.2) in a sterile homogenizer of stomacher type. Prepare sterile dilutions up to 0.000 001 using this homogenate.

B-2.3 Transfer aseptically dilutions beginning with 1 ml of 0.01 dilution and ending with 0.000 001 dilution into sterile petri dishes.

B-2.4 Introduce nearly 10 ml of the melted and cooled (40°C) agar (B-1.1) into the petri dishes (B-2.3) and mix by rotating gently.

B-2.5 Incubate the petri dishes (B-2.4) after setting at 37°C for 48 h. Count the colonies in the appropriate dilutions and compute their number per gram.

ANNEX C

[*Table* 2, *Sl No.* (ii)]

DETERMINATION OF *ESCHERICHIA COLI* COUNT

C-1 MEDIUM

C-1.1 Sterile Tergitol

7 Agar with the following composition shall be used:

Proteose peptone No. 3 Difco	5	g
Yeast extract	3	g
Lactose	10	g
Agar	15	g
Tergitol-7	0.1	ml
Bromothymol blue	0.025	g
Distilled water	1	litre

C-1.1.1 Dissolve the agar in three-fourths of the distilled water by steaming. Dissolve the other ingredients mentioned above (C-1.1) except Tergitol-7 and bromothymol blue in the rest of the distilled water by warming on water bath, if required. Mix the two solutions well, adjust the *p*H to 7.0 and filter through absorbent cotton. Add the Tergitol-7 and bromothymol blue in the filterate and mix well. Distribute in appropriate quantities and sterilize in an autoclave at 121°C for 15 minutes.

C-1.2 To every 100 ml of molten agar, cooled to a temperature of 40°C, add 0.4 ml of 1.0 percent solution of 2, 3, 5 triphenyl tetrazolium chloride prior to pouring into sterile petri dishes.

C-1.3 Dry the dishes (C-1.2) at 45°C for one hour.

C-1.4 To the surface of the dried agar (C-1.3) add 0.5 ml of 0.1 dilution of sample (*see* B-2.2) and spread it using a sterile bent glass rod.

C-1.5 Incubate the plates at 37°C for 18 to 24 h and count the characteristic yellow coloured colonies. Compute the number per gram.

ANNEX D

[*Table* 2, *Sl No.* (iii)]

DETERMINATION OF FAECAL *STREPTOCOCCI* COUNT

D-1 MEDIUM

D-1.1 Sterile KF agar with the following composition shall be used:

Proteose peptone No. 3	10	g
Yeast extract	10	g
Sodium chloride AR	5	g
Sodium glycerophosphate	10	g
Maltose CP	20.0	g
Lactose	1	g
Sodium azide	0.49	g
Sodium carbonate AR	0.063 6	g
Bromocresol purple	0.015	g
Agar	10	g
Distilled water (*see* IS 1070)	1	litre

D-1.1.1 Dissolve the agar in 750 ml of the distilled water by steaming. All ingredients mentioned above (D-1.1) except bromocresol purple and sodium carbonate are dissolved separately in 250 ml of distilled water. Mix the two solutions well. Add sodium carbonate in small portions and then filter through absorbent cotton. Add Bromocresol purple in the filterate and mix well. Distribute in appropriate quantities and sterilize in an autoclave at 121°C for 15 minutes.

NOTE — In case compounded agar medium is used, follow manufacturer's instructions for sterilization.

D-1.2 Add 1 ml of 1.0 percent solution of 2, 3, 5 triphenyl tetrazolium chloride per every 100 ml of the melted and cooled agar prior to use.

D-1.3 Pour 1 ml each of the 0.1 and 0.01 dilution sample (B-2.2) to two separate sterile petri dishes. Add the cooled agar (nearly 10 ml). Mix it by rotating. Incubate the plates at 37°C for 48 h. Count the red and pink colonies and compute their number per gram.

ANNEX E

[*Table* 2, *Sl No.* (iv)]

DETERMINATION OF COAGULASE POSITIVE *STAPHYLOCOCCI*

E-1 MEDIUM

E-1.1 Sterile Baired parker medium of the following composition shall be used:

Tryptone	10 g
Beef extract	5 g
Yeast extract	1 g
Lithium chloride	5 g
Agar	15 g
Distilled water (*see* IS 1070)	1 litre

E-1.1.1 Dissolve the agar in 750 ml of the distilled water (*see* IS 1070) by steaming. Dissolve the other ingredients mentioned above (E-1.1) in 250 ml of the distilled water by warming on a water bath, if required. Mix the two solutions well and adjust the *p*H to 7.0 followed by filtering hot through cotton. Distribute the filterate into conical flasks in quantities of 90 ml and sterilize in an autoclave at 121°C for 15 minutes.

NOTE — In case compounded agar medium is used, follow manufacturer's instructions for sterilization.

E-1.1.2 To every 90 ml of melted and cooled (40°C) medium, add successively the following sterile solutions:

Glycine, 20 percent	7 ml
Potassium tellurite, 1 percent	1 ml
Sodium pyruvate, 20 percent	5 ml
Egg yolk emulsion (yolk from one 5-ml egg is aseptically removed and mixed with 80 ml of sterile phosphate buffer)	

Mix well and pour in sterile petri dishes in 10 ml to 15 ml quantities. Allow to solidify and dry at 45°C for 1 hour.

E-2 PROCEDURE

E-2.1 To the surface of the dried agar, inoculate 0.5 ml of 0.1 dilution of the sample and spread it using a sterile bent glass rod. Incubate at 37°C for 24 h and count the black colonies surrounded by a clear zone of hydrolysis as coagulase positive *staphylococci* and compute their number per gram. In doubtful cases further confirmation may be done using plasma coagulation test.

E-2.2 Plasma Coagulation Test

Take 0.2 ml of citrated rabbit or human blood plasma in sterile test tube and dilute it with 0.6 ml of 0.4 percent sodium chloride solution in distilled water. Inoculate one loopful of a young culture of the organism under test and incubate at 37°C. Watch the tube at the interval of 30 minutes. Definite clotting of the plasma within 6 h indicates that the organism under test is coagulase positive.

ANNEX F

[*Table* 2, *Sl No.* (v)]

DETECTION OF *SALMONELLA*/ *ARIZONA*

F-1 PRINCIPLE

The sample is enriched in a nutritious non-selective medium followed by enrichment in a growth promoting medium containing selectively inhibitory reagents. The organism is then identified by biochemical and serological methods.

F-2 APPARATUS

a) Sterile petri dishes.

b) Sterile pipettes 1 ml, 10 ml with 0.1 ml graduation.

c) Inoculating needle and loop (3 mm).

d) Sterile culture tubes and tube racks.

F-3 MEDIA AND REAGENTS

a) Lactose Broth,
b) Selinite Cystine Broth,
c) Tetrathionate Broth,
d) Brilliant Green Agar (BGA),
e) Xylose Lysine Desoxycholate Agar (XLD),
f) Hektoen Enteric Agar (HEA),
g) Bismuth Sulphite Agar (BSA),
h) Triple Sugar Iron Agar (TSIA),
j) Tryptone Broth,
k) Urea Broth,
m) Malonate Broth,
n) Lysine Iron Agar (LIA),
p) Lactose, Sucrose, Salicin and Dulcitol in Andrades Fermentation Base,
q) Ortho Nitrophenyl Beta D. Galacto Pyranoside (ONPG) Broth,
r) Gram Stain Reagents,
s) Physiological Saline,
t) Hydrochloric Acid (1 N),
u) Sodium Hydroxide (1 N),
v) Kovac's Reagent, and
w) *Salmonella* Polyvalent Somatic 'O' Antiserum.

F-4 ISOLATION OF *SALMONELLA*

F-4.1 Pre-enrichment

Transfer aseptically 25 g of homogenized sample into a 500 ml conical flask containing 225 ml sterile lactose broth. Incubate at 37°C for 24 h.

F-4.2 Selective Enrichment

Transfer 1 ml each from **F-4.1** to 9 ml of selenite cystine broth and 9 ml Tetrathionate broth and incubate at 37°C for 24 h.

F-4.3 Streak loopful of culture from both the tubes of **F-4.2** to separate sterile petri dishes of Brilliant Green Agar (BGA), Hektoen Enteric Agar (HEA), Xylose Lysine Desoxycholate Agar (XLD) and Bismuth Sulphite Agar (BSA). Incubate the petri dishes for 24 h at 37°C and observe for characteristic Salmonella colonies after completion of incubation as given below:

BGA — Light pink transparent colonies. Surrounding media red.
HEA — Green-Blue colonies with black centre.
XLD — Red colonies with black centre.
BSA — Black colonies with silvery white metallic sheen.

F-4.4 Streak and stab all the typical *Salmonella* colonies from the plates of **F-4.3** (maximum 10) on Triple Sugar Iron Agar (TSIA) slants. Incubate for 24 h at 37°C and examine the tubes after incubation is complete. *Salmonella*, if present, will give either of the following reactions on TSI agar slants mentioned below:

a) Acid butt (yellow) and alkaline slant (red), gas and H_2S.
b) Acid butt, alkaline slant, no gas.

F-4.5 If the reactions in **F-4.4** are positive for *Salmonella*, perform the biochemical tests (*see* **F-4.6**).

F-4.6 Biochemical Tests

Media	*Incubation Time and Temp*	*Characteristic Salmonella*	*Reaction Arizona*
Lactose Broth	37°C / 24 h	No acid, no gas	Acid and gas (late)
Sucrose Broth	"	"	No acid, no gas
Salicin Broth	"	"	"
Dulcitol Broth	"	Acid and gas	"
Urea Broth	"	No colour change	No colour change
Lysine Iron Agar	"	Purple throughout	Purple throughout
Malonate Broth	"	No change	Blue colour
Ortho Nitrophenyl Beta D. Galacto Pyranoside Broth	"	No change	Yellow colour

F-5 SEROLOGICAL IDENTIFICATION

Isolates that give characteristic biochemical tests for *Salmonella/Arizona* are tested with *Salmonella* polyvalent Somatic 'O' anteserum in conjunction with a saline control. Isolates that agglutinate with *Salmonella* polyvalent 'O' anteserum and also give typical biochemical reactions are confirmed as *Salmonella /Arizona*.

ANNEX G

[*Table 2, Sl No.* (vii)]

IDENTIFICATION OF *V. CHOLERAE*

G-1 PRINCIPLE

G-1.1 The isolation of *Vibrio cholerae* and other biochemically related vibrios is based on the rapid growth of these organisms in an alkaline peptone water in the presence of available oxygen. Under such conditions *V. cholerae* and related vibrios will outgrow most of the contaminating pathogenic micro-organisms. They are then identified by biochemical and serological tests.

G-2 APPARATUS

a) Sterile petridishes;

b) Sterile pipettes — 1 ml, 5 ml, 10 ml with 0.1 ml graduation;

c) Inoculating needle and loop (3 mm);

d) Sterile culture tubes and tube racks; and

e) *p*H meter.

G-3 MEDIA AND REAGENTS

a) Alkaline Peptone Water;

b) Thiosulphate Citrate Bile Salt Sucrose Agar (TCBS);

c) Kligler Iron Agar (KIA);

d) Hugh-Liefson Glucose Broth;

e) Glucose, Sucrose, Mannitol, Inositol and Arabinose in Andrades Fermentation base;

f) Lysine-Ornithine Decarboxylase/Arginine-Dehydrolase Broths;

g) Gram Stain Reagents;

h) Physiological Saline;

j) Hydrochloric Acid (1 N);

k) Sodium Hydroxide (1 N); and

m) *V. cholerae* Polyvalent '01' Antisera.

G-4 ISOLATION OF *V. CHOLERAE*

G-4.1 Primary Enrichment

Transfer aseptically 25 g of homogenized sample in to a 500 ml conical flask containing 225 ml alkaline peptone water. Incubate at 37°C for 16 to 18 h. Streak one loopful of the inoculam from the surface growth on to TCBS Agar and incubate the plate at 37°C for 18 to 24 h.

G-4.2 Secondary Enrichment

Transfer 1 ml of inoculam from G-4.1 to 9 ml alkaline peptone water. Incubate at 37°C for 6 to 8 h. Streak one loopful of the inoculam from the surface growth on to TCBS Agar and incubate the plate at 37°C for 18 to 24 h.

G-4.3 Examine the TCBS plates from G-4.1 and G-4.2 for *V. cholerae* colony characteristics. *V. cholerae* will appear as large, smooth yellow transparent colonies with translucent peripheries.

G-4.4 Inoculate all the individual suspected colonies but to a maximum of 10 in to Kligler Iron Agar (KIA) media by stabbing the butt and streaking the surface of slant. Incubate the inoculated slants at 37°C for 18 to 24 h.

Examine the KIA slants. *V. cholerae* will produce acid butt (yellow in colour) and alkaline slant (red in colour) and will not produce gas and hydrogen sulphide.

G-4.5 Biochemical Tests

G-4.5.1 With the suspicious colonies perform the following biochemical reactions, with the undermentioned media:

Media	*Incubation Temperature and Time*	*Typical Reaction of V. Cholerae*
Hugh Liefson	37°C/24 h	Motile and fermentative
Glucose Broth	do	Acid, no gas
Sucrose Broth	do	do
Mannitol Broth	do	do
Inositol	do	No acid, no gas
Arabinose Broth	do	do
Lysine Broth	37°C 1-4 days	Purple colour
Ornithine Broth	do	do
Arginine Broth	do	Yellow colour
Gram Stain	do	Gram Negative comma shape

G-5 SEROLOGICAL IDENTIFICATION

G-5.1 Isolates that give characteristic biochemical tests are tested with *V. cholerae* polyvalent 01 antiserum in conjunction with a saline control.

Cultures that agglutinate in 01 antiserum and also give typical biochemical reactions are confirmed as *Vibrio cholerae* 01.

Cultures that do not agglutinate with 01 antiserum but give typical biochemical reactions are grouped as *Vibrio cholerae* non 01 or non-agglutinable vibrios.

ANNEX H

[*Table* 2, *Sl No.* (viii)]

IDENTIFICATION OF *L. MONOCYTOGENES*

H-1 PRINCIPLE

The sample is first enriched in a suitable enrichment medium followed by streaking on presumptive media. Presumptive colonies developed after incubation at 30°C for 24 to 48 h are confirmed by biochemical reactions.

H-2 APPARATUS

a) Binocular Research Microscope;
b) Stomacher Homogenizer;
c) Sterile Petridishes;
d) Conical Flasks;
e) 1 ml Graduated Pipettes·
f) Test Tubes;
g) Sugar Fermentation Tubes, Durham Tubes;
h) Platinum Wire with Holder;
j) Microscopic Slides; and
k) Cavity Slides.

H-3 MEDIA/REAGENTS NEEDED

a) Oxoid Listeria Enrichment Broth Base Containing Oxoid Listeria Primary Selective Enrichment Supplement.
b) Oxoid Listeria Selective Agar Base Containing.Listeria Selective Supplement.
c) SIM Media
d) Triple Sugar Iron Agar (TSIA)
e) Urea Agar Slants
f) Dextrose, Maltose, Esculin, Mannitol, Rhamnose Xylose Broths
g) Crystal Violet, Grams Iodine, Ethyl Alcohol, Saffranine, etc, for Gram's Reaction
h) Nutrient Agar Slants

H-4 PROCEDURE

H-4.1 Enrichment

Inoculate 25 g sample in 225 ml of Oxoid Listeria enrichment broth base containing Oxoid Listeria primary selective enrichment supplement. Incubate for 24 h at room temperature.

H-4.2 Selective Media

Streak one loopful from enrichment medium after incubation (*see* H-4.1) to plates of Oxoid Listeria selective agar base containing Oxoid Listeria selective supplement and incubate at room temperature for 24 to 48 h.

H-4.2.1 *Listeria Monocytogenes*

Listeria monocytogenes will grow as purple brown transparent colonies changing the colour of the surrounding media to purple brown.

H-4.3 Stab typical colonies to SIM media and incubate at room temperature for 48 h. *L. monocytogenes* will exhibit umbrella motility.

H-4.4 Biochemical Tests

With the suspicious colonies perform the following biochemical reactions, with the undermentioned media:

Media	*Incubation Temperature and Time*	*Typical Reaction of L. Monocytogenes*
Gram Stain		Gram + rods
Motility	2-3 days/30° C	Tumbling motility
TSI Agar	24 h - 48 h/37° C	Acid butt and slant no gas
Fermentation in Dextrose broth	do	Acid and no gas
Fermentation in Maltose broth	do	do
Fermentation in Esculin broth	do	do
Fermentation in Rhamnose broth	do	do
Fermentation in Mannitol broth	do	No acid no gas
Fermentation in Xylose broth	do	do

ANNEX J

[*Table* 2, *Sl No.* (ix)]

DETERMINATION OF FORMALDEHYDE

J-1 PRINCIPLE

Quantitative determination of formaldehyde involves extraction of the fish muscle tissue with trichloroacetic acid for the removal of proteins and treatment with acetyl acetone — ammonium acetate reagent to form a coloured formaldehyde derivative. The absorbance is measured by spectrophotometer.

J-2 REAGENTS

a) Trichloroacetic acid (10 percent solution);

b) Sodium hydroxide (30 percent solution);

c) Acetic acid (5 percent solution); and

d) Acetyl Acetone — ammonium acetate reagent. To prepare 1 litre of the reagent, dissolve 150 g of ammonium acetate, 3 ml of glacial acetic acid and 2 ml of acetyl acetone in distilled water (*see* IS 1070) and make up volume to 1 litre.

J-3 EXTRACT

Mince the fish muscle tissue using a homogenizer. Macerate 10 g of the fish mince in 10 percent trichloroacetic acid for 2 minutes using a mortar and pestle and then filter through a filter paper (Whatman No. 42) and make up the volume to 100 ml in a standard volumetric flask.

J-4 PROCEDURE

Place 5 ml trichloroacetic acid extract of the tissue in a 50 ml beaker. Add 10 ml of distilled water (*see* IS 1070) and make the solution alkaline with a few drops of 30 percent NaOH and adjust the *p*H to 6.0 with 5 percent acetic acid. Make up the solution to 25 ml with water and mix 5 ml of it with 5 ml of acetyl acetone — ammonium acetate reagent. Keep the mixture still for 50 minutes at 37°C and read the colour at a wave length of 410 'nm'.

For a blank, take distilled water in duplicate in the place of sample and for standard, prepare a series of tubes using 100 μg formaldehyde standard solution, concentration ranging from 0-80 μg.

J-5 CALCULATIONS

$$\text{Formaldehyde (mg, percent)} = \frac{\text{Absorbance of sample} \times \text{Concentration in standard} \times 25 \times 100 \times 100}{\text{Absorbance of standard} \times 5 \times 5 \times 10 \times 1\,000}$$

ANNEX K

[*Table* 2, *Sl No.* (x)]

DETERMINATION OF INDOLE

K-1 PRINCIPLE

Indole is extracted with light petroleum from trichloroacetic acid — precipitated shrimp muscle. The extracted indole, soluble in light petroleum, is reacted and re-extracted with Ehrlich's reagent. Indole in the form of a rose indole complex can be determined spectrophotometrically.

K-2 APPARATUS

a) Spectrophotometer, and

b) Refrigerated centrifuge.

K-3 REAGENTS

a) Trichloroacetic Acid (TCA) — Dissolve 6 g TCA in 100 ml distilled water.

b) Light petroleum (Boiling point 40-60°C).

c) Ehrlich's Reagent — Dissolve 9 g paradimethylaminobenzaldehyde (PABA) in 45 ml concentrated HCl in 250 ml volumetric flask and dilute to volume with ethanol.

d) Standard Indole Solutions — Accurately prepare stock solution of 10 mg indole in 100 ml light petroleum. Use 1:10 dilution as working solution. Refrigerate indole solutions.

K-4 PROCEDURE

K-4.1 Homogenize 40 g shrimp with 80 ml ice-cold trichloroacetic acid solution (TCA) in a waring blender for 1 minute. Add 80 ml ice-cold light petroleum and blend fof 1 minute. Transfer homogenate to 250 ml centrifuge bottle and centrifuge for 10 minutes at 10 000 rev/min. Filter supernate through Whatman No. 1 paper under suction. Transfer filtrate to 250 ml separatory funnel. After the two layers have separated, tansfer acid layer (lower) to second 250 ml separatory funnel.

K-4.2 Wash TCA

Denatured protein precipitate (separated by centrifugation) with 40 ml light petroleum and filter as described above. Transfer filtrate to second 250 ml separatory funnel already containing TCA layer from first extraction. Shake for 1 minute and let two layers separate. Transfer lower acid layer to third separatory funnel and extract for third time with 40 ml light petroleum.

K-4.3 Combine all light petroleum extracts into 1 separatory funnel. Extract indole with exactly 5 ml freshly prepared Ehrlich's reagent by vigorously shaking for 1 minute. The rose indole complex formed is quantitatively transferred to Ehrlich's reagent layer. When layers have separated, transfer lower layer to 1 cm path cell and read at 570 nm against reagent black solution.

K-4.4 Prepare standard curve as follows: Accurately measure volumes from 0.5 to 4 ml stock indole solution (working solution) and add into 80 ml TCA in a separatory funnel. Extract indole by procedures described above and construct standard curve. Rose indole complex from indole standard and from TCA-extracted shrimp is stable up to 4 h.

K-5 CALCULATION

With the help of the standard curve the amount of indole present in 40 g shrimp can be determined. Indole content is usually expressed as the amount of indole in microgram per 100 g shrimp muscle.

ANNEX L

[*Table* 2, *Sl No.* (xi)]

DETERMINATION OF HEAVY METALS

L-1 PRINCIPLE

The sample is digested with nitric acid and sulphuric acid (mineralisation) in a closed system. The heavy metals are then determined by Atomic Absorption Spectrophotometer. Mercury is determined using cold vapour technique and arsenic after generation of metal hydrides.

L-2 PREPARATION OF MATERIAL

Preparation of sample of fish involves inclusion of skin and exclusion of bones, but subject to overall rule of edibility. This material is thoroughly minced to get a homogeneous sample. This material is used for the determination of trace metals.

L-3 DETERMINATION OF MERCURY IN FISH

L-3.1 Principle

Mercury present in fish muscle is converted into ionic mercury by wet oxidation using concentrated nitric acid and concentrated sulphuric acid in the ratio 4:1. The solution containing Hg^{2+} ions are reduced to elemental mercury using stannous chloride. The liberated mercury vapour is measured using a Mercury Analyser, in the cold state.

L-3.2 Reagents Required

L-3.2.1 *Stannous Chloride* ($SnCl_2$) *Solution*, (20 *percent* m/v) — Dissolve 20 g stannous chloride ($SnCl_2$) in 10 ml distilled hydrochloric acid (HCl) and boil it for about a minute; cool and dilute to 100 ml. Add 1 to 2 g of tin metal after the preparation of the solution.

L-3.2.2 *Potassium Dichromate* ($K_2Cr_2O_7$) *Solution (1 percent* m/v *in Water)*

L-3.2.3 *Potassium Permanganate* (KMnO4) *Solution (5 percent* m/v *in Water)* — $KMnO_4$ solution tends to absorb mercury from ambient atmosphere if kept exposed. Hence the bottle must be kept stoppered.

L-3.2.4 *Nitric Acid* (HNO_3) (10 *percent* v/v) — Prepare in distilled water (*see* IS 1070).

L-3.2.5 *Sodium Hydroxide* (NaOH) *Solution* (20 *percent* m/v) — Dissolve 50 g of sodium hydroxide pellets in distilled water (*see* IS 1070) and dilute to 250 ml.

L-3.2.6 *Sulphuric Acid* (H_2SO_4) (1:1) — Prepare by diluting with distilled water (*see* IS 1070).

L-3.2.7 *Standard Mercury Solution*

L-3.2.7.1 *Stock solution* — Dissolve 0.135 4 g of mercuric chloride ($HgCl_2$) in 25 ml of 5 percent nitric acid (HNO_3). Add 1 ml potassium dichromate ($K_2Cr_2O_7$) and make up the volume to 100 ml with 5 percent nitric acid (HNO_3).

L-3.2.7.2 *Working standard* — 25 μg, 50 μg, 100 μg, 150 μg and 200 μg per 5 ml or 10 ml solution by successive dilution of the above standard. Maintain 5 percent nitric acid (HNO_3) and 0.01 percent potassium dichromate ($K_2Cr_2O_7$) in the solution.

L-3.3 Digestion Procedure for the Determination of Mercury

Weigh about 5 to 10 g of wet material (homogenized) and transfer to the oxidation flask followed by the addition of a cold mixture of concentrated nitric acid (HNO_3) and concentrated sulphuric acid (H_2SO_4) in the ratio of 4:1 (v/v). It is heated cautiously at first, collecting the distillate in the reservoir. When the mixture starts to darken, add a little of the distillate into the flask. The procedure is continued, maintaining a slight excess of nitric acid (HNO_3) in the oxidation flask, until the solution ceases to darken and fumes of SO_3 are evolved. The solution is cooled and make up to a known volume using distilled water (*see* IS 1070).

L-3.4 Determination of Mercury Using Mercury Analyser

A suitable aliquot of the sample (5 to 10 ml) is pipetted into the reaction vessel of the apparatus and the required amount of 10 percent nitric acid (HNO_3) solution is added in order to maintain total volume of 10 ml or 20 ml. Add 2 ml of stannous chloride ($SnCl_2$) solution and stopper immediately. Stir the contents vigorously for 5 minutes. The absorbance is noted on the meter as early as possible. The sample procedure is adopted with a series of standards and reagent blank. The reaction vessel is thoroughly cleaned after each measurement. Standard curve is prepared using mercuric chloride ($HgCl_2$).

L-4 DETERMINATION OF COPPER AND ZINC

L-4.1 Apparatus

L-4.1.1 Atomic Absorption Spectrophotometer with hollow cathode lamps.

L-4.1.2 *Air-Acetylene Fame*

L-4.2 Reagents

L-4.2.1 *Concentrated Nitric Acid* (HNO_3) — redistilled.

L-4.2.2 *Perchloric Acid* ($HClO_4$) — 70 percent double vacuum distilled.

L-4.2.3 *Concentrated Sulphuric Acid* (H_2SO_4).

L-4.3 Copper Standard Solution

L-4.3.1 *Stock Solution* (1 mg/ml) — Place 0.200 g copper wire or foil into a 125 ml Erlenmeyer flask. Add 15 ml nitric acid (1:4), cover flask with watch glass and let copper dissolve, warming to complete solution. Boil to expel fumes, cool and dilute to 200 ml with distilled water (*see* IS 1070).

L-4.3.2 *Working Standard* (Range 2 to 8 μg/ml) — Prepare required concentrations daily, using sulphuric acid (2N H_2SO_4).

L-4.4 Zinc Standard Solution

L-4.4.1 *Stock Solution* (1 mg/ml) — Dissolve 1 g Zinc powder in 20 ml hydrochloric acid (1:1) in 1 litre volumetric flask and dilute to volume with distilled water (*see* IS 1070).

L-4.4.2 *Working Standard* — (0.2 μg/ml, 0.5 μg/ml, 1.0 μg/ml and 1.5 μg/ml). Pipette 1 ml stock solution into 100 ml volumetric flask and make up the volume with distilled water. From this solution suitably dilute to get the required concentration.

L-4.5 Digestion of the Sample (Wet Oxidation)

L-4.5.1 Weigh 25 g of sample into a suitable size kjeldahl flask and add 10 to 15 ml concentrated nitric acid (HNO_3) and keep overnight for pre-digestion. Heat cautiously until first vigorous reaction subsides somewhat; then add 5 ml concentrated sulphuric acid (H_2SO_4). Continue heating, adding more concentrated nitric acid (HNO_3) in small portions as needed to prevent charring, until fumes of SO_3 evolve and solution remains clear and almost colourless. Add 0.5 ml perchloric acid ($HClO_4$) and continue heating until it is almost completely removed. Excess nitric acid is destroyed by adding saturated ammonium oxalate solution to the cooled, digested solution. It is evaporated again to get SO_3 fumes. The solution is cooled and diluted to a known volume using distilled water (*see* IS 1070).

L-4.5.2 The metals are determined directly by aspirating the solution over the flame in an atomic absorption spectrophotometer and the corresponding readings are noted. Calibration of the instrument is carried out using standard solutions of copper and zinc. Air acetylene flame is used for atomisation.

L-5 DETERMINATION OF ARSENIC BY HYDRIDE GENERATION METHOD

L-5.1 Principle

The vapour generation method works by reacting the analyte element in an acidified solution with sodium borohydride. The resulting metal hydrides are relatively unstable and decompose at low temperature.

L-5.2 Apparatus

L-5.2.1 *Atomic Absorption Spectrophotometer*

L-5.2.2 *Vapour Generation Assembly*

L-5.3 Reagents

L-5.3.1 *Acids for Digestion* (*see* L-4.2.1 to L-4.2.3)

L-5.3.2 *Sodium Borohydride Pellets*

L-5.3.3 *Potassium Iodide Solution* — Dissolve 20 g KI in 100 ml distilled water (*see* IS 1070).

L-5.3.4 *Arsenic Standard Solution*

L-5.3.4.1 *Stock solution* — Dissolve 1.320 g As_2O_3 in minimum volume of 20 percent sodium hydroxide (NaOH) in 1 litre volumetric flask, acidify with hydrochloric acid (1:1) and dilute to make up volume to 1 litre with distilled water (*see* IS 1070).

L-5.3.4.2 *Working standard* — (1 μg/ml, 2 μg/ml, 3 μg/ml, 4 μg/ml and 5 μg/ml). Pipette 10 ml stock solution into 100 ml volumetric flask and dilute to volume with water. Pipette 1 ml, 2 ml, 3 ml, 4 ml and 5 ml of above diluted solution into separate 100 ml volumetric flasks and make up the volume with distilled water (*see* IS 1070).

L-5.4 Digestion of Sample (Wet Oxidation)

Weigh 25 g of sample into a suitable size kjeldahl flask and add 10 to 15 ml concentrated nitric acid (HNO_3) and keep overnight for pre-digestion. Heat cautiously until first vigorous reaction subsides somewhat; then add 5 ml concentrated sulphuric acid (H_2SO_4). Continue heating, adding more concentrated nitric acid (HNO_3) in small portions as needed to prevent charring, until fumes of SO_3 evolve and solution remains clear and almost colourless. Add 0.5 ml perchloric acid ($HClO_4$) and continue heating until it is almost completely removed. Excess nitric acid is destroyed by adding saturated ammonium oxalate solution to the cooled, digested solution. It is evaporated again to get SO_3 fumes. The solution is cooled and diluted to a known volume using distilled water (*see* IS 1070).

L-5.5 Determination

L-5.5.1 Add 5 ml of hydrochloric acid (HCl) and 5 ml distilled water (*see* IS 1070) to 10 ml of working solution to make up 20 ml of solution. Arsenic must be reduced to Arsenic (III), by the addition of 1 ml of 20 percent m/v potassium iodide (KI) solution to each aliquot of sample. Reduction takes 1 h at room temperature or 5 minutes at 50°C. Cool the solution to ambient temperature before analysis.

L-5.5.2 The sample is placed in the reaction vessel. Light the flame and adjust the fuel for a blue flame. Turn the stirrer on and wait for 20 seconds. Dispense 5 ml sodium borohydride via the septum. Allow the reaction to complete. The hydride formed is carried from the reaction vessel by a flow of inert gas (N_2) and thermally decomposed in a quartz tube heated by an acetylene flame.

L-6 DETERMINATION OF LEAD

L-6.1 Apparatus (*see* L-5.2)

L-6.2 Reagents (*see* L-5.3)

L-6.3 Lead Standard Solution

L-6.3.1 *Stock Solution* (1 mg/ml) —Dissolve 1 g lead powder in 20 ml nitric acid (1:1) in 1 litre volumetric flask, and dilute to volume with distilled water (*see* IS 1070).

L-6.3.2 *Working Standard* (5 μg/ml, 10 μg/ml, 15 μg/ml to 20 μg/ml). Pipette 1 ml, 2ml, 3ml and 4 ml stock solutions into 200 ml volumetric flasks respectively and make up the volume with distilled water (*see* IS 1070).

L-6.4 Digestion Procedure

Dry the homogenized sample and make it into a powder. Take 1 to 2 g material in a silica crucible and place in a furnace at 250°C. The temperature is slowly raised to 350°C with holding at this temperature till smoking ceases. The temperature is then increased to 500°C and the sample is ashed at this temperature for 16 h. The carbon free ash is cooled and dissolved in 5 ml of nitric acid (1 N HNO_3). It is then warmed on a hot plate to aid dissolution and is made up to 50 ml in a standard flask using distilled water (*see* IS 1070).

The solution is aspirated over the flame of Atomic Absorption Spectrophotometer and the concentration is measured.

L-7 DETERMINATION OF TIN

L-7.1 Principle

Samples are digested with nitric acid and then hydrochloric acid and are diluted with distilled water. Aqueous potassium chloride is added to samples and standards. Tin is determined by atomic absorption spectrophotometer at 235.5 nm with oxidizing acetylene flame.

L-7.2 Apparatus

L-7.2.1 *Atomic Absorption Spectrophotometer (AAS)*

L-7.3 Reagents

L-7.3.1 *Nitric Acid* (HNO_3)

Test purity of lot by diluting with distilled water (1:4 v/v) and aspirating into atomic absorption spectrophotometer. Absence of tin signal indicates suitability for analysis.

L-7.3.2 *Potassium Chloride Solution*

Dissolve 1.91 g KCl and dilute to 100 ml with distilled water (*see* IS 1070).

L-7.3.3 *Tin (Sn) Standard Solution*

L-7.3.3.1 *Stock solution* — (1 mg /ml). Dissolve 1 g of tin (reagent grade) metal in 200 ml concentrated hydrochloric acid (HCl). Add 200 ml of distilled water, cool and make up the volume to 1 litre.

L-7.3.3.2 *Working standard* — (0 µg/ml, 50 µg/ml, 100 µg/ml, 150 µg/ml and 200 µg/ml). Pipette 5 ml, 10 ml, 15 ml or 20 ml of stock solution into 100 ml volumetric flasks respectively and add 10 ml concentrated hydrochloric acid (HCl) and 1.0 ml (potassium chloride) KCl solution. Make up the volume with distilled water (*see* IS 1070).

L-7.4 Digestion of Sample

L-7.4.1 Weigh accurately about 25 g of sample in an Erlenmeyer flask and dry in oven at 120°C. Add 30 ml concentrated nitric acid (HNO_3) into the flask and within 15 minutes heat gently to initiate digestion (avoid excessive frothing). Boil gently till samples just begin to dry at the bottom (avoid charring). Add 25 ml concentrated hydrochloric acid (HCl) and heat gently for 15 minutes until evolution of chlorine gas stops. Boil until solution reduces to 10 to 15 ml. Add 40 ml water, swirl, and pour into 100 ml volumetric flask. Conduct two reagent blanks.

L-7.4.2 Pipette 1.0 ml potassium chloride (KCl) solution into above 100 ml volumetric flask. Cool to ambient temperature and make up the volume with distilled water (*see* IS 1070). Mix well and filter through medium porosity paper.

L-7.4.3 Aspirate water before and after each sample. Calibrate the instrument using tin standard and read the concentration of the sample.

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